Structure-Function Studies of Peptides Inhibiting the Ribonucleotide Reductase Activity of Herpes Simplex Virus Type I[1]

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Ac-Tyr[298]-Ala[299]-Gly[300]-Thr[301]-Val[302]-Ile[303]-Asn[304]-Asp[305]-Leu[306]-OH (Ac-VZV R2-(298-306)) represents the acetylated form of the C-terminus of varicella-zoster virus (VZV) ribonucleotide reductase subunit 2 (R2). This peptide possesses a high degree of homology with the C-terminus nonapeptide of the herpes simplex virus (HSV) type I and II ribonucleotide reductase R2 protein and is 15 times more potent than the latter in its in vitro inhibition of HSV-1 reductase activity. Accordingly, a new series of analogues based on this structure was studied in vitro. The replacement of Asp[305] by Asn, Glu, Gln, Ser, or Cys; of Asn[304] by Gln or Ser; of Ile[303] and Val[302] by D-Val; and of Tyr[298] by Cha induced an important loss of inhibitory potency. The substitution of Asn[304] by Asp; of Thr[301] by Cys, Ser, or Val; of Gly[300] by Ala or Val; of Ala[299] by Val; or of Tyr[298] by homoPhe, 4′-fluoro-Phe, 4′-chloro-Phe, 3′-iodo-Tyr, Me-Tyr, or For-Trp led to a moderate decrease of the Ac-VZV R2-(298-306) potency. The replacement of Val[302] by Ile; Ala[299] by Cys, Ser, or Thr; or the insertion of a six- or eight-carbon chain between Tyr[298] and the NH_2 terminus either preserved or slightly increased the inhibitory potency of Ac-VZV R2-(298-306). Finally, the substitution of Tyr[298] by Trp or the addition of 4′-nitro-Phe at the amino terminus resulted in a 3-fold increase of potency. Altogether, these results stress the importance of the structural integrity of the minimum active core 302-306 in preserving the inhibitory potency and suggest that further studies on monosubstitutions could be directed at the portion 298-301 of the peptide.

Introduction

Viruses of the herpes family code for their own ribonucleotide reductase (RR),[2-7] an enzyme catalyzing the reduction of ribonucleoside diphosphates to their 2′-deoxy forms. Like their eukaryotic and prokaryotic counterparts, the herpes RRs consist of two nonidentical subunits (R1 and R2) that must associate to form the catalytic site.[8] Recent studies on herpes simplex virus type I (HSV-1) RR have stressed the importance of this enzyme in HSV DNA replication.[9-11] Therefore, disruption of the subunit interaction may constitute a new target for antiviral chemotherapy. This strategy originated from the observation that the synthetic peptide H-Tyr[329]-Ala[330]-Gly[331]-Ala[332]-Val[333]-Val[334]-Asn[335]-Asp[336]-Leu-[337]-OH, HSV R2-(329-337),[32] inhibits herpes simplex virus type I and II (HSV-2),[12,13] pseudorabies,[14] and equine herpes virus[15] RRs. This structure, which corresponds to the C-terminal portion of both HSV-1 and HSV-2 R2, is completely inactive against mammalian RRs.[12,13,16] The inhibition of HSV-1 RR is produced by the specific binding of HSV R2-(329-337) to the HSV-R1 protein, which prevents the association of the two subunits.[17,18]

In a previous study on the structure-activity relationship, we have documented that the disubstitution of Ala[332] by Thr and Val[334] by Ile, which exists in the native VZV R2 C-terminus nonapeptide (VZV R2-(298-306)),[32] and that Nα-acetylation enhance 4- and 3-fold, respectively, the potency of HSV R2-(329-337) to inhibit, in vitro, the RR activity of HSV-1.[19] Furthermore, combination of both modifications has a synergetic effect, generating a peptide 15 times more potent than HSV R2-(329-337).[20] In addition, we have recently demonstrated that Ac-VZV R2-(298-306) is resistant to in vitro proteolysis.[20] Thus, Ac-Tyr[298]-Ala[299]-Gly[300]-Thr[301]-Val[302]-Ile[303]-Asn[304]-Asp[305]-Leu[306]-OH so far represents the best compound to use for further extending our previous work on the structure-function relationship of RR inhibitors[19] and to explore structural modifications that could improve Ac-VZV R2-(298-306) potency. We now report the synthesis and the RR inhibitory potency of 35 analogues of this peptide, focusing on the importance of amino acid side chain

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(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (*Eur. J. Biochem.* 1984, *158*, 9-37). All optically active amino acids are of the L configuration, unless otherwise specified. Additional abbreviations are used: Ac, acetyl; Cha, cyclohexylalanyne; For, formyl; homoPhe, 2-amino-4-phenylbutyric acid; Me, methyl; Z, benzyloxycarbonyl.

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Table I. Physicochemical Data[e]

no.	MW	FAB-MS[a]	% overall yield	HPLC t_R,[b] min	HPLC % homogeneity (214 nm/280 nm)
1	1007		31	23.0,[c] 16.7[d]	99.7/99.9[c,d]
2	1006		56	21.8,[c] 20.7[d]	99.7/99.8[c,d]
3	1021		22	24.0,[c] 17.2[d]	96.5/96.4[c,d]
4	1020		22	21.6,[c] 20.6[d]	98.5/99.0[c,d]
5	979		12	23.4,[c] 21.0[d]	98.5/99.9[c,d]
6	995	995	39	27.4,[c] 23.6[d]	96.5/96.2,[c] 96.7/96.6[d]
7	1021		76	24.4,[c] 17.3[d]	99.8/99.5[c,d]
8	980		45	24.6,[c] 17.4[d]	97.9/97.2[c,d]
9	1008		64	24.2,[c] 14.9[d]	99.8/97.9,[c] 99.9/97.3[d]
10	993		52	20.0,[c] 16.6[d]	97.3/97.2[c,d]
11	1007		15	25.6,[c] 19.0[d]	97.0/99.8[c,d]
12	1021		18	26.6,[c] 18.0[d]	99.9/99.7[c,d]
13	1009	1011	14	25.2,[c] 17.8[d]	99.5/99.7[c,d]
14	993		22	23.2,[c] 16.4[d]	98.0/97.0[c,d]
15	1005		8	25.8,[c] 18.4[d]	98.9/99.8[c,d]
16	1021		13	25.8,[c] 17.4[d]	96.8/97.4[c,d]
17	1049		13	27.6,[c] 19.0[d]	97.0/96.8[c,d]
18	1035		9	26.6,[c] 18.2[d]	98.2/99.4[c,d]
19	1039	1040	48	23.8,[c] 17.0[d]	99.8/99.2[c,d]
20	1023		63	21.2,[c] 16.0[d]	99.8/99.7[c,d]
21	1037		72	27.8,[c] 16.9[d]	98.7/99.9,[c] 99.5/97.7[d]
22	997		34	33.6,[c] 22.6[d]	99.0/[c,d]
23	1005		14	30.4,[c] 21.2[d]	99.7/[c,d]
24	1009		42	29.6,[c] 20.2[d]	99.8/[c,d]
25	1026		7	31.8,[c] 22.0[d]	96.8/[c,d]
26	1133		5	28.2,[c] 22.0[d]	99.8/99.7[c,d]
27	1259		10	31.6,[c] 20.6[d]	99.5/99.6[c,d]
28	1021		50	27.2,[c] 18.6[d]	98.9/99.3[c,d]
29	1030	1030	22	23.0,[c] 20.6[d]	96.9/97.0[c,d]
30	1058	1056	46	29.8,[c] 21.9[d]	99.8/99.0[c,d]
31	1050		21	23.2,[c] 17.4[d]	99.8/99.4[c,d]
32	1078		19	25.0,[c] 18.8[d]	99.5/99.6[c,d]
33	1106		10	28.2,[c] 21.4[c]	99.8/99.7[c,d]
34	1199		45	30.4,[c] 22.3[d]	99.9/99.6[c,d]
35	1037		25	23.4,[c] 17.9[d]	99.2/99.9[c,d]
36	1108		20	23.2,[c] 19.9[d]	99.0/99.3[c,d]

[a] (M + 1) ± mass unit. [b] t_R, retention time. [c] Linear gradient; solvent A consisted of 0.01% aqueous TFA (pH 2.9), and solvent B consisted of CH_3CN–0.01% TFA, 0.75% B/min for 40 min, initial condition, 10% B, flow rate 1.5 mL/min, 23 °C. [d] Linear gradient; solvent A consisted of 0.01 M aqueous NH_4OAc (pH 6.9), and solvent B consisted of CH_3CN, 1.0% B/min for 40 min; initial condition, 0% B, flow rate 1.5 mL/min, 23 °C. [e] Satisfactory amino acid analysis obtained.

function and/or size in positions 298, 299, 300, 301, 304, and 305 and on the effect of N-terminus elongation.

Results and Discussion

Peptides 1–36 were synthesized by solid-phase methodology using a scheme based on *t*-Boc chemistry and acid-labile amino acid protecting groups, as used in our previous work on RR inhibitors.[19] However, BOP[21] was used as coupling reagent with concomitant neutralization[22,23] instead of couplings with preformed symmetrical anhydrides. This approach allowed 4 times less consumption of amino acid derivatives and shorter cycles without affecting overall peptide yield and quality.

Purification, accomplished in one step by reverse-phase high-performance liquid chromatography (HPLC), yielded homogeneous products (≥97%) as shown in Table I. Amino acid analyses of the pure peptides after acidic hydrolysis confirmed their theoretical composition (data not shown). The molecular mass of cysteinyl- (**6, 13, 19**) and tryptophyl-containing peptides (**29, 30**) was also validated by fast atom bombardment/mass spectroscopy (FAB-MS) (Table I).

Inhibition of HSV-1 RR activity by peptides **1–36** is reported in Table II. Analogues **2–12** comprise monosubstitutions at the C-terminal portion (302–306) of Ac-VZV R2-(298–306). It was previously shown that it represents the minimum active core of HSV R2-(329–337).[19] We have also reported that substitutions of Leu[337] by various aliphatic or aromatic amino acids either decreased (Val, Ile) or abolished (Ala, Phe, Tyr) the inhibitory activity. In the present study, substitution at Leu[306] was not further explored. The replacement of Asp[305] by Asn, Glu, Gln, Ser, or Cys (compounds **2–6**) induced a drastic decrease of potency (RI ≤ 0.8%). This indicates that the carboxylic side chain must have a precise length and cannot be extended by one carbon atom. Moreover, the free carboxylic function cannot be substituted by a carboxamide, an hydroxyl, or a thiol, suggesting that the carboxylate ion of Asp[305] may either participate directly in the binding to the R1 subunit or may elicit a conformation that favors optimal binding of the peptide to R1. Similarly, the substitution of Asn[304] by Gln (**7**) or Ser (**8**) led to an important loss of potency (RI ≤ 5%) while a substitution by Asp (**9**) resulted in a compound that retained 41% of Ac-VZV R2-(298–306) activity, stressing the importance of the carboxylate for the maintenance of inhibitory potency. We previously observed, in the HSV series, that a reduction of the hydrophobic character in position 334 or 333, by substituting Val by Ala, led to an important loss of inhibitory potency (RI ≤ 8%).[19] An increase of hydrophobicity in position 334 also had a deleterious effect on potency (RI = 41%).[19] In addition, the substitution of Ala[332] by a prolyl residue did not significantly change the peptide potency (RI = 92%),[19] sug-

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Table II. Inhibitory Potency of Peptide Analogues of the C-Terminus VZV Subunit 2 Ribonucleotide Reductase[a]

no.	compound	primary structure	IC_{50},[b] μM	% RI[c]
		329 330 331 332 333 334 335 336 337		
	HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	36–60[19]	
		298 299 300 301 302 303 304 305 306		
1	Ac-VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	3.2	100
2	Ac-[Asn^{305}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-Ile-Asn-**Asn**-Leu	640 (3.9)[d]	0.6
3	Ac-[Glu^{305}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-Ile-Asn-**Glu**-Leu	650 (3.0)	0.5
4	Ac-[Gln^{305}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-Ile-Asn-**Gln**-Leu	345 (2.7)	0.8
5	Ac-[Ser^{305}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-Ile-Asn-**Ser**-Leu	>1000 (2.0)	
6	Ac-[Cys^{305}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-Ile-Asn-**Cys**-Leu	>1000 (1.7)	
7	Ac-[Gln^{304}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-Ile-**Gln**-Asp-Leu	73 (3.6)	5
8	Ac-[Ser^{304}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-Ile-**Ser**-Asp-Leu	395 (2.7)	0.7
9	Ac-[Asp^{304}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-Ile-**Asp**-Asp-Leu	8.7 (3.6)	41
10	Ac-[D-Val^{303}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-Val-**D-Val**-Asn-Asp-Leu	>1000 (2.3)	
11	Ac-[D-Val^{302}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-**D-Val**-Ile-Asn-Asp-Leu	>1000 (2.3)	
12	Ac-[Ile^{302}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-Thr-**Ile**-Ile-Asn-Asp-Leu	2.6 (3.3)	127
13	Ac-[Cys^{301}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-**Cys**-Val-Ile-Asn-Asp-Leu	3.9 (3.3)	85
14	Ac-[Ser^{301}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-**Ser**-Val-Ile-Asn-Asp-Leu	5.1 (3.1)	61
15	Ac-[Val^{301}]VZV R2-(298–306)	Ac-Tyr-Ala-Gly-**Val**-Val-Ile-Asn-Asp-Leu	3.7 (3.1)	83
16	Ac-[Ala^{300}]VZV R2-(298–306)	Ac-Tyr-Ala-**Ala**-Thr-Val-Ile-Asn-Asp-Leu	14.3 (3.3)	23
17	Ac-[Val^{300}]VZV R2-(298–306)	Ac-Tyr-Ala-**Val**-Thr-Val-Ile-Asn-Asp-Leu	4.6 (3.3)	72
18	Ac-[Val^{299}]VZV R2-(298–306)	Ac-Tyr-**Val**-Gly-Thr-Val-Ile-Asn-Asp-Leu	4.2 (2.3)	55
19	Ac-[Cys^{299}]VZV R2-(298–306)	Ac-Tyr-**Cys**-Gly-Thr-Val-Ile-Asn-Asp-Leu	3.1 (3.3)	106
20	Ac-[Ser^{299}]VZV R2-(298–306)	Ac-Tyr-**Ser**-Gly-Thr-Val-Ile-Asn-Asp-Leu	2.2 (3.1)	140
21	Ac-[Thr^{299}]VZV R2-(298–306)	Ac-Tyr-**Thr**-Gly-Thr-Val-Ile-Asn-Asp-Leu	3.6 (4.5)	125
22	Ac-[Cha^{298}]VZV R2-(298–306)	Ac-**Cha**-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	11.3 (1.9)	17
23	Ac-[HomoPhe^{298}]VZV R2-(298–306)	Ac-**HomPhe**-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	30 (1.9)	6
24	Ac-[4′-fluoro-Phe^{298}]VZV R2-(298–306)	Ac-**4′-fluoro-Phe**-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	5.4 (1.9)	35
25	Ac-[4′-chloro-Phe^{298}]VZV R2-(298–306)	Ac-**4′-chloro-Phe**-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	5.6 (2.5)	44
26	Ac-[3′-iodo-Tyr^{298}]VZV R2-(298–306)	Ac-**3′-iodo-Tyr**-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	2.8 (2.5)	89
27	Ac-[3′,5′-iodo-Tyr^{298}]VZV R2-(298–306)	Ac-**3′,5′-iodo-Tyr**-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	8.0 (2.6)	33
28	Ac-[Me-Tyr^{298}]VZV R2-(298–306)	Ac-**Me-Tyr**-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	12.1 (6.7)	55
29	Ac-[Trp^{298}]VZV R2-(298–306)	Ac-**Trp**-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	1.9 (5.9)	313
30	Ac-[N^{in}-For-Trp^{298}]VZV R2-(298–306)	Ac-**For-Trp**-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	13.8 (4.1)	30
31	[4-aminobutyryl^{297}]VZV R2-(297–306)	H2N-**C4**-Tyr-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	2.7 (2.1)	78
32	[6-aminohexanoyl^{297}]VZV R2-(297–306)	H2N-**C6**-Tyr-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	1.7 (2.1)	118
33	[8-aminooctanoyl^{297}]VZV R2-(297–306)	H2N-**C8**-Tyr-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	1.9 (2.1)	110
34	Ac-[4′-nitro-Phe^{297}]VZV-(297–306)	Ac-**4′-nitro-Phe**-Tyr-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	1.5 (4.2)	280
35	Ac-[Ser^{299},Ile^{302}]VZV R2-(298–306)	Ac-Tyr-**Ser**-Gly-Thr-**Ile**-Ile-Asn-Asp-Leu	4.3 (4.6)	107
36	[6-aminohexanoyl^{297},Ser^{299},Ile^{302}]VZV R2-(297–306)	H2N-**C6**-Tyr-**Ser**-Gly-Thr-**Ile**-Ile-Asn-Asp-Leu	8.5 (4.6)	54

[a] Results are expressed in percentage of the HSV-1 ribonucleotide reductase activity obtained in control experiments without peptide and represents the mean of two or three determinations that varied less than 10% with each other. [b] IC_{50}, concentration of peptide producing 50% of the control value. [c] RI, relative inhibition in percentage compared to Ac-VZV R2-(298–306). [d] Respective IC_{50} of Ac-VZV R2-(298–306), in each experiment.

gesting that a conformational constraint might be tolerated or even beneficial in position 303 or 302 of the VZV peptide. However, the introduction of a D-valine in position 303 or 302 (**10**, **11**) probably led to a conformational change that did not allow the interaction of Ac-VZV R2-(298–306) with the R1 subunit, resulting in a complete loss of potency. Substitution of Val^{302} by an isoleucyl residue (**12**) had slightly less influence on the inhibitory potency of Ac-VZV R2-(298–306) (RI = 127%) than to that of Val^{334} on HSV R2-(329–337) (RI = 148%),[19] indicating that an increase of hydrophobicity is less effective in position 302 than in position 303.

Peptides **13**–**21** represent monosubstitutions in the spacer region 299–301. It was previously reported, in the HSV series, that such a distance was necessary between the minimum active core and the N-terminus tyrosyl residue to preserve optimal inhibitory potency.[19] Moreover, in the same study the substitution of Ala^{332} for Thr increased the inhibitory potency of HSV R2-(329–337) 1.5-fold.[19] To extend our studies on the structure–activity relationship at this position, Thr was replaced by Cys (**13**), Ser (**14**), or Val (**15**). These substitutions had little influence on the inhibitory potency of Ac-VZV R2-(298–306) (RI = 61–85%). Replacement of Gly^{300} by Ala (**16**) resulted in an analogue 4 times less potent than **1**, while increasing hydrophobicity and steric bulk by Val (**17**) restored the potency. Previously, we had shown that the substitution of this glycyl residue by Pro slightly increased the inhibitory potency (RI = 119%).[19] Altogether, these results suggest that a certain molecular constraint imposed by Val or Pro could be favorable for the interaction of Ac-VZV R2-(298–306) with the R1 subunit. Substitution of Ala^{299} by a branched aliphatic residue such as Val (**18**) led to a slight loss of potency, while replacement by functionalized amino acids such as Cys (**19**), Ser (**20**), or Thr (**21**) restored or increased the potency 1.2–1.4-fold, indicating that the presence of a free hydroxyl such as in position 301 could be beneficial.[19]

It has been reported that the N-terminus tyrosyl residue contributed to increase the inhibitory potency of the peptide through its aromatic character and its free hydroxylic function.[19] We have extended these data with analogues **22**–**34**. Replacement of the Tyr^{298} by a cyclohexylalanyl (**22**) or a homophenylalanyl (**23**) residue decreased the inhibitory potency of Ac-VZV R2-(298–306) (RI = 6–17%) to a level comparable to that of the HSV fragment lacking the N-terminal tyrosyl residue (RI = 13%).[19] This indicates that the aromatic character is essential and that the phenyl ring must protrude at a critical distance to influence inhibitory potency. Replacement of the hydroxyl group on Tyr^{298} by fluorine (**24**) or chlorine (**25**), which have strong electron-withdrawing inductive effects, may again induce conformational changes that cause a greater loss of potency (RI = 35–44%) than that created by removing the hydroxyl function ([Phe^{329}]HSV R2-(329–337); RI = 61%).[19] Iodination of Tyr^{298} in position

3′ of the ring (**26**) slightly decreased the potency of Ac-VZV R2-(298–306) (RI = 89%), whereas in positions 3′ and 5′ (**27**) it resulted in a 3.1-fold reduction. Therefore, the monoiodotyrosyl analogue could be used as a radioligand in the development of a binding assay to directly test the affinity of Ac-VZV R2 analogues for the R1 subunit. Substitution of the Tyr[298] by an *N*-methyl tyrosyl residue (**28**) probably induced some steric hindrance and/or conformational change that influenced the orientation of the phenolic hydroxyl moiety, reducing the potency of this analogue (RI = 55%) almost to that of the [D-Tyr[329]]HSV R2-(329–337) analogue (RI = 30%).[19] The introduction of an indole ring (Trp) in position 298 (**29**) increased the potency of Ac-VZV R2-(298–306) 3.1 times. Addition of an *N*-formyl group on the indole ring (**30**) has been shown to sometimes enhance potency.[24] However, in the present nonapeptide structure this had the opposite effect, suggesting that the unprotected nitrogen heteroatom favors binding to the R1 subunit.

Finally, replacement of the *N*-acetyl group, an acylation that substantially increased inhibitory potency of HSV R2-(329–337) and VZV R2-(298–306),[19,20] by an aminobutyryl, aminohexanoyl, or aminooctanoyl moiety (**31–33**) to increase peptide hydrophobicity slightly affected Ac-VZV R2-(298–306) potency (RI = 78–118%). Addition of an Ac-4′-NO_2-phenyl residue (**34**) at the N-terminus of VZV R2(298–306), a N-terminus elongation that increased 7.8-fold the inhibitory potency of HSV R2-(329–337),[18] had less effect on the VZV nonapeptide although it increased its potency 2.8-fold. Selected monosubstitutions that increased the inhibitory potency of Ac-VZV R2-(298–306), such as Ser[299], Ile[302], and 6-aminohexanoyl[297], resulted in a decreased potency when combined (**35**, **36**), indicating that only subtle changes can be made to improve the inhibitory potency.

In conclusion, results from the present structure–activity study together with those from our previous work[19] indicate that the minimum active cores, VZV R2-(302–306) and HSV R2-(333–337), play a crucial role in the maintenance of inhibitory potency, while the N-terminal portion better tolerates monosubstitution and could be the site of further modifications to improve the RR inhibitory potency. Among the most potent analogues (**12**, **20**, **29**, **34**), the Trp[298]-nonapeptide structure Ac-Trp-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu, which is 3.1 times more potent than Ac-VZV R2-(298–306) and 45 times more potent than HSV R2-(329–337), constitutes a new lead. Replacement of Tyr 298 by substituted indole or indene rings will now be needed to further assess its relevance in developing a new generation of RR inhibitors.

Experimental Section

Materials. *p*-(Chloromethyl)poly(sterene-*co*-divinylbenzene 1%) resin (0.65 mequiv of chlorine/g) and N^{α}-(*tert*-butyloxycarbonyl) (Boc) protected amino acid derivatives were purchased from Bio-Méga Inc. and IAF Biochemicals. Amino acid side chains were protected as follows: Asp, β-O-Bzl; Glu, γ-O-Bzl; Thr, O-Bzl; Ser, O-Bzl; Cys, *S*-4-MeBzl; Tyr, *O*-2-Br-Z; (Me)Tyr, *O*-2,6-Cl_2-Z; 3′-iodo-Tyr, *O*-3-Br-Z; 3′,5′-diiodo-Tyr, *O*-3-Br-Z; Trp, N^{in}-For. Prior to use, all amino acid derivatives were tested for purity by thin-layer chromatography and melting point determination. N^{α}-Boc-8-aminooctanoic acid, N^{α}-Boc-6-aminohexanoic acid, N^{α}-Boc-4-aminobutyric acid, and Boc-4′-nitro-Phe[18] were N^{α}-acylated by means of di-*tert*-butyl dicarbonate.[25,26] Reagent-grade 2-propanol and methylene chloride (CH_2Cl_2) were brought from Anachemia Canada Inc. CH_2Cl_2 was distilled from anhydrous sodium carbonate. *N,N*-Diisopropylethylamine (DIEA) (Aldrich Chemicals) was distilled from ninhydrin and subsequently kept at 4 °C. *N,N*-Dimethylformamide (DMF) (Anachemia Canada Inc.) was distilled from ninhydrin, in vacuo, after a 3-day storage over 4-Å molecular sieves and kept under an argon atmosphere. Anisole was distilled and kept at 4 °C. Trifluoroacetic acid (TFA) (Halocarbon Products Co.) and acetic acid (HOAc) (Anachemia Canada Inc.) were distilled prior to use. *N,N′*-Dicyclohexylcarbodiimide (DCC), acetic anhydride (Ac_2O) (Aldrich Chemicals), 1-hydroxybenzotriazole (HOBT), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (Richelieu Biotechnologies), Accusolv-grade methanol (MeOH), and acetonitrile (CH_3CN) (Anachemia Canada Inc.) were used without further purification.

Peptide Synthesis. Merrifield resin[27] was coupled to Boc-Leu, by using the cesium salt method.[28] The degree of substitution was determined by the picric acid colorimetric test[29] and was 0.41 mmol of amino acyl/g. Compounds **1–36** were prepared by automated solid-phase synthesis (Vega 250). Couplings of Boc amino acid derivatives (3 equiv), except for Boc-Asn and Boc-Gln, were achieved with BOP,[21–23] using in situ neutralization (6 equiv DIEA). The latter two (3 equiv) were coupled with DCC/HOBT. The coupling completion was ascertained by a ninhydrin colorimetric test.[30] Boc protecting groups were removed with TFA–CH_2Cl_2 (40:60) containing 1% D,L-methionine when Cys or Trp were present in the peptide chain followed by a neutralization with DIEA–CH_2Cl_2 (5:95) when the DCC/HOBT method was used. After completion of the synthesis and removal of the last Boc group, the peptides were N-acetylated with Ac_2O. Deprotection of the amino acid side chains and cleavage of the peptides from the resin were performed with anhydrous hydrogen fluoride (HF) and anisole (9:1, v/v; 10 mL/g of peptide-resin intermediate) at −15 °C for 30 min and then at 0 °C for an additional 30 min. When Cys or Trp was present in the peptide chain, 0.5% D,L-methionine (w/v) was added to the reaction mixture. N^{in}-For was removed in situ with HF, anisole, and 1,2-ethanedithiol (85:10:5, v/v/v).[31] HF removal was done in vacuo followed by precipitation of the crude peptides with peroxide-free anhydrous ethyl ether and solubilization with 20% aqueous N_2-purged HOAc. Solutions were lyophilized to yield amorphous powders.

Peptide Purification and Physicochemical Characterization. The crude peptides were examined using analytical HPLC to optimize the purification procedure. Then, a sample load ranging from 100 to 150 mg was subjected to preparative HPLC on a Partisil 10 ODS-3 Whatman (10-μm particle size) column (2.2 cm × 50 cm) using a binary solvent system consisting of 0.01

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M aqueous ammonium acetate (NH_4OAc) (pH 6.9) and CH_3CN and appropriate gradients. A flow rate of 7.0–8.0 mL/min was used. Elution of the peptides was monitored at 214 and/or 280 nm. Collected fractions were readily screened by analytical HPLC and pooled accordingly. The peptides thus obtained were subjected to rotary evaporation, in vacuo, to remove CH_3CN and then lyophilized twice. Purified peptides were analyzed for homogeneity by analytical HPLC on a μBondapak C_{18} (0.39 × 15 cm, 10-μm particle size) column using appropriate linear gradients of 0.01% aqueous TFA (pH 2.9) and 0.01% TFA–CH_3CN and of 0.01 M ammonium acetate (pH 6.9) and CH_3CN. Their amino acid composition and peptide content were assessed by quantitative amino acid analysis after acidic hydrolysis in vacuo (6 N HCl, 110 °C, 18 h), as we previously described.[19] Individual amino acid recovery ranged from 0.83 to 1.08/residue, except for Cys and Trp. The molecular mass of peptides **6**, **13**, **19**, **29**, and **30** and therefore Cys and Trp integrity were assessed by FAB-MS on a Kratos MS-50 TATC instrument.

HSV-1 Ribonucleotide Reductase Assay. The inhibitory effect of the synthetic peptides, on HSV-1 RR activity, was determined as previously described[19] using HSV-1 RR partially purified from quiescent BHK-21/C13 cells infected with strain F at 20 plaque-forming units per cell. The specific activity of the viral reductase preparation was 37 units/mg protein, one unit of RR being defined as the amount of enzyme generating 1 nmol of deoxycytidine 5'-diphosphate per hour under the assay conditions.

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Bis Basic Substituted Diaminobenzobisthiazoles as Potential Antiarthritic Agents

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A series of benzobisthiazoles were screened for antiinflammatory activity in the carrageenan paw edema and adjuvant arthritis tests. Compound **26**, 2,6-bis(*N*,*N*-diethylamino)benzo[1,2-*d*:5,4-*d*']bisthiazole, was found to inhibit the swelling of the uninjected paw in the prophylactic adjuvant arthritis model with an ED_{50} of 2.3 mg/kg orally. As with most compounds of this series, **26** was inactive in acute model of inflammation, such as paw edema; like steroids, it showed activity in the granuloma pouch assay but did not inhibit cyclooxygenase, indicating a mode of action different from the classical nonsteroidal antiinflammatory drugs (NSAID's). At doses higher than those producing antiinflammatory activity, **26** had some immunoregulating properties.

Ever since it became clear that the classical nonsteroidal antiinflammatory drugs (NSAID's) produced gastrointestinal side effects by virtue of cyclooxygenase (CO) inhibition, research in medicinal laboratories has been directed at finding compounds which interfere with the underlying cause of the arthritic diseases. The name given to these elusive agents, "disease modifying agents" (DMA's), indicates the vagueness of the concept. One avenue of research was to concentrate on the aberrant autoimmune reaction believed to be the result of an inflammatory stimulus of unknown origin.[1]

At the onset of the present work, very few chemicals were known to possess a selective mode of action on either the humoral or the cellular arms of the immune system.[2] One such experimental agent was tilorone (**90a**). Originally found to be an interferon inducer,[3] it was later shown to suppress cell-mediated responses and, in contrast, to enhance antibody production in animals models.[4] The compound was also reported to suppress adjuvant induced arthritis in rats[4–6] and experimental allergic encephalomyelitis,[4,6] two cell-mediated, delayed type reactions.

Tilorone has since generated an active search for bioisosteric analogues with an improved biological profile (for review see ref 7). In our laboratories, we have examined, as a potential source of antiarthritic drugs, the benzobisthiazole systems having as basic side chains (alkylamino)acetamido or related functions in lieu of the alkylamino ether moiety of tilorone. The compounds were screened in the acute paw edema and the chronic adjuvant arthritis assays. In the latter test, high activity in suppressing the secondary inflammation in the uninjected paw was considered to be the result of interference with the immune response.[8]

Chemistry

Basic [(alkylamino)acyl]amino side chains were introduced into the diaminobenzobisthiazoles I, II, III, IV, and V ($Y_1 = Y_2 = NH_2$) (Tables I and II). Two general methods were used: (1) chloroacylation with excess chloroacyl chloride (or anhydride) followed by treatment of the resulting chloroacetylamines with appropriate al-

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